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Original Article

Impact of HBV and HCV coinfection on CD4 cells among HIV-infected patients: a longitudinal retrospective study

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Abstract

Introduction: The impact of hepatitis B virus (HBV) and hepatitis C virus (HCV) coinfection on CD4 cells in patients with human immunodeficiency virus (HIV) is unclear. We aimed to examine the impact of HBV and HCV coinfection on CD4 cell count and CD4/CD8 ratio in adults with HIV.

Methodology: We conducted a longitudinal retrospective study in Brazil between January 1, 2002, and June 30, 2016, including 205 patients with HIV monoinfection, 37 with HIV-HBV coinfection, 35 with HIV-HCV coinfection, and 62 with HIV-HCV (48 HCV genotype 1 and 14 HCV genotype 3).

Results: Median duration of follow-up was 2,327 (interquartile range: 1,159–3,319) days. An increased CD4 cell count and CD4/CD8 ratio over time was observed in all groups receiving combined antiretroviral therapy (cART). Patients with HIV-HBV or HIV-HCV coinfection and those with HIV monoinfection, showed comparable CD4 cell counts and CD4/CD8 ratios during pre-ART. There was also no statistically significant difference in CD4/CD8 ratio between HIV-HBV or HIV-HCV coinfection groups and the HIV monoinfection group during follow-up on cART. However, CD4 cell counts were significantly lower in HIV-HCV patients than in HIV monoinfection patients during follow-up on cART. HIV patients with HCV genotype 3 coinfection showed significantly lower CD4/CD8 ratio during follow-up on cART than those coinfected with HCV genotype 1 coinfection. No statistically significant effect of coinfection was observed on the efficacy of cART.

Conclusions: HIV-infected patients are more likely to show better immunological responses to cART when they are not coinfected with HCV.

Key words: CD4 lymphocyte count; coinfection; HIV; hepatitis B virus; hepatitis C virus.

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Introduction

Human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) are serious pathogens that substantially contribute to the global burden of disease [1,2]. It has been estimated that 36.7 million people worldwide are living with HIV infection [1], 257 million people with chronic HBV infection, and 71 million people with HCV infection [2]. These viruses share common routes of transmission and many people with HIV are coinfected with HBV and/or HCV [3,4]. In this context, an estimated 2.7 million people have been reported with HIV-HBV coinfection, and 2.3 million with HIV-HCV coinfection [2].

HBV and HCV infections among HIV-infected individuals are of utmost importance owing to the severe outcomes of coinfection [5]. Studies have reported that the risk of liver-related mortality is 2–3 times higher in HIV-HBV-coinfected patients than in HIV patients [6,7]. Data have demonstrated that patients with HIV-HCV coinfection are hospitalized more often and have longer hospital stays than those with HIV monoinfection. In addition, HCV-related end-stage liver disease has been established as a leading cause of in-hospital mortality among HIV-infected patients [8]. Combination antiretroviral therapy (cART) may attenuate these risks [9]; however, cART and hepatitis virus reactivation are associated with liver toxicity in HIV-coinfected individuals, rendering them more susceptible to liver-related diseases and rapid progression of Acquired Immune Deficiency Syndrome (AIDS) [10,11]. Consequently, HIV, HBV, and HCV coinfections have become public health concerns.

The impact of HBV and HCV is not limited to causing liver disease, but it also results in failure to

achieve immunological recovery in patients with HIV infection [12,13]. However, the effect of HBV and HCV on the CD4 cell count in HIV-infected patients remains a topic of much controversy. The large number of investigations conducted in these patients have yielded conflicting findings, with some studies reporting a negative effect on CD4 cells [14,15] and others reporting no effect [16,17]. To clarify these questions, we compared the mean CD4 cell count and the CD4/CD8 ratio between HIV-monoinfected and HBV- or HCV-coinfected patients in pre-ART and during follow-up on cART. To our knowledge, this is the first study in Brazil to use a linear mixed-effects model to evaluate the impact of HBV and HCV on the CD4 cell count in HIV-infected individuals.

Methodology

Study area

The study was conducted at two reference centers for the diagnosis, treatment, and follow-up of HIV/AIDS and viral hepatitis: The Specialized Center for Parasitic Infectious Diseases (CEDIP) belonging to 10ª Regional de Saúde (10th Regional Health) in the city of Cascavel, and the Specialized Service of Sexually Transmitted Infections of the Secretary of Health belonging to 15ª Regional de Saúde (15th Regional Health) in the city of Maringá, both in Paraná State, Southern Brazil. These two centers serve 25 and 30 municipalities, respectively, with a total population of 1,189,062 [18] and are part of the Sistema Único de Saúde (SUS; the Unified Health System). The treatment program for patients with HIV infection at these two reference centers consists of follow-up visits with health care professionals for treatment pickup and monitoring of the HIV viral load level, CD4 and CD8 cell count; other laboratory indices (e.g., liver and kidney function, lipid profile) and clinical data are also recorded.

Study design

Three longitudinal retrospective studies were performed between January 1, 2002 and June 30, 2016 to determine potential differences in CD4 cell counts, CD4/CD8 ratio, and HIV viral load between the following groups: patients with HIV monoinfection *versus* HIV-HBV coinfection; patients with HIV monoinfection *versus* HIV-HCV coinfection; and HIV-coinfected patients with HCV genotype 1 *versus* genotype 3. Patients were diagnosed with HBV infection if they were positive for hepatitis B surface antigen (HBsAg) and negative for anti-HCV or HCV RNA. Patients who were negative for HBsAg and positive for both anti-HCV and HCV RNA were diagnosed with HCV infection. Participants were diagnosed with HIV monoinfection when they were negative for HBsAg, anti-HCV, and HCV RNA. For the HIV monoinfection, HIV-HBV, and HIV-HCV groups, the inclusion criteria were as follows: (1) patients with HIV only for the HIV monoinfection group, and patients with HIV-HBV or HIV-HCV coinfection for the coinfected groups, at baseline (first entry into the data set for each patient); (2) age ≥ 18 years at baseline; (3) adherence to cART during follow-up; and (4) at least two measurements of CD4 cell counts and CD4/CD8 ratio during the study period; (5) patients who were ART-naive (baseline) and then started a cART regimen, including at least three antiretroviral drugs.

Participants were followed up until June 30, 2016 or until: patient death, or discontinuation of cART, or loss to follow-up. To increase the power of the study, a ratio of 2.70 HIV monoinfection to 1 coinfected patient was used for the HIV-HBV group (100 HIV monoinfection patients), and 3 HIV monoinfection to 1 coinfected patient for the HIV-HCV group (105 HIV monoinfection patients). HIV monoinfection and HIV-coinfected groups were paired according to the follow-up time on cART; a variation of up to four months between groups was allowed. For the comparison between HCV genotypes 1 and 3 among HIV infection patients, criterion 3 (adherence to cART during follow-up) was not applied, as it would further reduce the small number of patients coinfected with HCV genotype 3. Patients with HCV genotypes 1 and 3 were selected because they are the most prevalent groups in the study region as well as in Southern Brazil [19].

Laboratory measurements

HIV infection status was based on positive test results for two peripheral blood samples using enzyme-linked immunosorbent assay (ELISA) (Abbott Diagnostics, Chicago, USA) and confirmed with western blot (Bio-Rad, Marnes La Coquette, France).

Seropositivity of HBsAg and anti-HCV antibody was determined with second- or third-generation ELISA techniques (Abbott Diagnostics, Chicago, USA). Positive results for the presence of anti-HCV were confirmed by amplification of HCV RNA using reverse transcription PCR (RT-PCR) (Roche Diagnostics, Basel-city, Switzerland).

Abbott RealTime HCV Genotype II (Abbott Laboratories, Chicago, USA) was used in the diagnostic routine activity to determine the HCV genotype based on dual-target real-time PCR: the 5'-UTR region

represented the target to discriminate between HCV genotypes, using previously described methods [20]. Viral genotype was determined after phylogenetic analysis of the sequences obtained, along with established GenBank reference sequences [21].

Plasma CD4 cell and CD8 cell counts were estimated with flow cytometry (BD Trucount™ Tubes, Franklin Lakes, New Jersey, USA) using the FACSCalibur apparatus (Becton-Dickinson, Franklin Lakes, New Jersey, USA) and the results were expressed in cells/mm^3. Plasma HIV RNA levels were measured using real-time polymerase chain reaction (Roche Diagnostics, GmbH, Mannheim, Germany) with a detection limit of 50 copies/ml. Data of CD4 cell count, CD4/CD8 ratio, and HIV viral load were stored and subsequently obtained for tabulation of data from the national network of the Sistema de Controle de Exames Laboratoriais (SISCEL; the Laboratory Test Control System), at the virology laboratory of the State University of Maringá. All information from SISCEL is stored using data encryption in its central database, which is located in the Department of Surveillance, Prevention and Control of STIs, HIV/AIDS and Viral Hepatitis of the Ministry of Health and can be accessed online [22].

Statistical analysis

Pearson's Chi-square test or Fisher's exact test were adopted for categorical variables and the Mann–Whitney test was used in terms of quantitative variables. Profiles for mean CD4 cell counts and CD4/CD8 ratios during follow-up were developed with a linear mixed-effects model, as described in the literature [23]. The mixed-effects model used to adjust for the square root values of CD4 counts and CD4/CD8 ratio included the fixed effects Time, Group and the interaction Time versus Group. In all models, the heterogeneous variance for the groups was also tested. The interaction was not significant between the groups, thus we only examined the main effects: Time and Group. Model selection was performed using the Akaike Information Criterion [24], Bayesian Information Criterion [25], and the Likelihood ratio test [26]. Graphical analysis of the residues was performed, and in the selected models, there was no serious violation of the normality hypothesis. Time-related analyses were reported in days; however, for graphical representation these were summarized in intervals of one year. To resolve this dispersion, the maximum follow-up period was 10 years.

To estimate the association between HIV viral load and the progression of the disease of each group studied, HIV viral load was divided into two categories (< 50 copies/mL and ≥ 50 copies/mL). This variable was adjusted for by means of a mixed logistic model with a random effect for each patient, taking into account the time of study and the group studied. Odds ratios (OR) with 95% confidence intervals (CI) were used to express the magnitude of association between categorical variables and a p-value < 0.05 was considered statistically significant. Statistical analysis was performed with the R version 3.3.3 statistical environment [27]. This study was reviewed and approved by the Research Ethical Committee of the University of Assis Gurgacz Foundation (Report No. 1.397.212 of 28/01/2016).

Results

During the study period, 3340 patients with HIV were diagnosed at both referral centers, of which 4.97% (166/3340) had HBV and/or HCV coinfection. Seroprevalence of HIV-HBV and HIV-HCV was 1.86% (62/3340) and 2.90% (97/3340), respectively. Only 0.21% (7/3340) of HIV-infected patients had triple infections (HIV-HBV-HCV). The age of patients with HIV coinfections ranged from 23 to 75 years, with a median age of 46 years and interquartile range (IQR) of 40–53 years. Among all coinfected patients, 63.25% (105/166) were men (median age: 42 years, IQR 42–53 years) and 36.75% (61/166) were women (median age: 45 years, IQR 37–53 years) ($p = 0.07$), showing a sex ratio of 1.72:1.

Of all HIV-infected patients diagnosed in the study period, 6.46% (205/3,174) of those with HIV monoinfection, 59.68% (37/62) of those with HIV-HBV coinfection, 36.08% (35/97) of those with HIV-HCV, and 63.91% (62/97) of those with HCV genotypes 1 and 3 among HIV-coinfected patients met the inclusion criteria for the longitudinal retrospective study. The median duration time of follow-up of all patients was 2,327 (IQR 1,159–3,319) days, with a median of 12 (IQR 7–19) laboratory measurements and an interval of 160 (IQR 123–201) days between measurements. In pre-ART (data at the first measurement after a positive anti-HIV test), a total of 191/205 (93.17%) HIV monoinfection patients, 35/37 (94.59%) HIV-HBV-coinfected patients, and 32/35 (91.42%) HIV-HCV-coinfected patients had an inverted CD4/CD8 ratio, with the following medians: 0.31 (IQR 0.21–0.55), 0.29 (IQR 0.21–0.60), and 0.29 (IQR 0.17–0.60), respectively. There was no statistically significant difference in CD4 cell count and CD4/CD8 ratio between the HIV monoinfection and HIV-HBV groups, or the HIV monoinfection and HIV-

HCV groups in pre-ART; there was also no statistically significant difference in CD4 cell counts and CD4/CD8 ratio between HIV coinfection patients with HCV genotypes 1 and 3 in pre-ART. There was also no significant difference between groups in pre-ART with respect to sociodemographic and risk behaviors, except for the higher proportion (29.73%; 11/37) of HBV-coinfected patients with more than five sexual partners in the last 12 months, compared with 11.00% (11/100) among patients with HIV monoinfection (p = 0.02), as well as the high frequency (34.29%;12/35) of illicit drug use among HCV-coinfected patients compared with 6.67% (7/105) of HIV monoinfection patients (p < 0.01). Baseline characteristics of paired groups in the longitudinal retrospective study are shown in Table 1.

There was a significant increase over the Time variable in the CD4 cell count and CD4/CD8 ratio for all groups of patients. (p < 0.01). There was no statistically significant difference between patients with HIV monoinfection and HIV-HBV in CD4 cell counts during follow-up on cART (p = 0.09). There was also no statistically significant difference in CD4 cell counts between HIV coinfection patients with HCV genotypes 1 and 3 (p = 0.23) (Table 2). There was no statistically significant difference in CD4/CD8 ratio between HIV monoinfection and HIV-HBV groups during follow-up on cART (p = 0.36); the same was true between HIV monoinfection and HIV-HCV groups (p = 0.28) (Table 3). However, we observed lower CD4 cell counts in the HIV-HCV group compared with the HIV monoinfection group during follow-up on cART (p = 0.03). Furthermore, CD4/CD8 ratio was significantly lower in HIV coinfection patients with HCV genotype 3 than HCV genotype 1 during follow-up (p = 0.04). There was no significant difference in HIV viral load between the HIV-HBV or HIV-HCV groups and their respective HIV monoinfection groups, as well as between HCV genotypes 1 and 3 (Table 4).

Table 1. Baseline characteristics of paired groups in longitudinal retrospective study.

Variables	HIV monoinfection n (%)	HIV-HBV n (%)	p-value[a]	HIV monoinfection n (%)	HIV-HCV n (%)	p-value[b]	HIV-HCV-1 n (%)	HIV-HCV-3 n (%)	p-value[c]
Age (median, IQR)	40.5 (35-49)	44 (37-54)	0.13	44 (35-52)	47 (41-55)	0.08	49 (42-53)	48 (45-54)	0.95
Gender									
Male	60 (60.00)	28 (75.68)	0.14	55 (52.38)	22 (62.86)	0.38	29 (60.42)	10 (71.43)	0.45
Female	40 (40.00)	9 (24.32)		50 (47.62)	13 (37.14)		19 (39.58)	4 (28.57)	
Ethnicity									
White	61 (61.00)	25 (67.57)	0.74	70 (66.67)	28 (80.00)	0.15	40 (83.33)	10 (71.43)	0.61
Black	5 (5.00)	2 (5.41)		3 (2.86)	2 (5.71)		2 (4.17)	1 (7.14)	
Brown	34 (34.00)	10 (27.03)		32 (30.48)	5 (14.29)		6 (12.50)	3 (21.43)	
Education									
≤ 8 years	58 (58.00)	23 (62.16)	0.66	64 (60.95)	23 (65.71)	0.76	31 (64.58)	8 (57.14)	0.61
> 8 years	42 (42.00)	14 (37.84)		41 (39.05)	12 (34.29)		17 (35.42)	6 (42.86)	
Sexual behavior									
Heterosexual	87 (87.00)	27 (77.14)	0.27	91 (86.67)	30 (85.71)	0.89	35 (72.92)	10 (71.43)	0.91
Homosexual/bisexual	13 (13.00)	8 (22.86)		14 (13.33)	5 (14.29)		13 (27.08)	4 (28.57)	
Number of sexual partners in last 12 months									
≤ 1	55 (55.00)	19 (51.35)	0.02	50 (47.62)	15 (42.86)	0.31	19 (39.58)	2 (14.29)	0.25
2-5	20 (20.00)	2 (5.41)		19 (18.10)	3 (8.57)		3 (6.25)	2 (14.29)	
> 5	11 (11.00)	11 (29.73)		14 (13.33)	5 (14.29)		12 (25.00)	6 (42.85)	
Not reported	14 (14.00)	5 (13.51)		22 (20.95)	12 (34.28)		14 (29.17)	4 (28.57)	
Use of illicit drugs									
Yes	11 (11.00)	6 (16.22)	0.41	7 (6.67)	12 (34.29)	< 0.01	15 (31.25)	4 (28.57)	0.85
No	89 (89.00)	31 (83.78)		98 (93.33)	23 (65.71)		33 (68.75)	10 (71.43)	
Pre-ART CD4 mean ± SD (cells/mm^3)	341 ± 216	402 ± 243	0.17	428 ± 288	372 ± 243	0.37	311 ± 184	316 ± 280	0.57
Pre-ART CD4/CD8 mean ± SD	0.36 ± 0.28	0.42 ± 0.36	0.45	0.45 ± 0.32	0.44 ± 0.40	0.91	0.38 ± 0.34	0.35 ± 0.31	0.50
Pre-ART HIV RNA mean ± SD (log 10 copies/mL)	3.84 ± 1.64	3.95 ± 1.49	0.70	3.66 ± 1.57	3.64 ± 1.99	0.51	3.82 ± 2.05	4.23 ± 1.59	0.81

HIV, human immunodeficiency virus; HBV, hepatitis B virus; HCV, hepatitis C virus; ART, antiretroviral therapy; n, number of patients; SD, standard deviation; IQR, interquartile range; Fisher's exact test or Pearson's Chi-square test were adopted for categorical variables and the Mann–Whitney test was used to compare the two groups in terms of quantitative variables; p-value[a] for comparison between HIV monoinfection and HIV-HBV groups; p-value[b] for comparison between HIV monoinfection and HIV-HCV groups; p-value[c] for comparison between HCV genotype 1 and 3 groups among HIV-infected.

Table 2. Estimate with standard errors for the fixed effects parameters of the model for CD4 cell counts.

Group	Parameters	Estimates	SE	LI	LU	p-valor
HIV-HBV	Intercept	19.1237	0.4765	18.1890	20.0058	< 0.01
	Time	0.0034	0.0003	0.0028	0.0034	< 0.01
	HIV-HBV	1.6456	0.9635	-0.2591	3.5503	0.09
HIV-HCV	Intercept	20.6015	0.5213	19.5792	21.6239	< 0.01
	Time	0.0019	0.0002	0.0014	0.0024	< 0.01
	HIV-HCV	-1.9635	0.9201	-3.7818	-0.1451	0.03
HIV-HCV-3	Intercept	18.8305	0.7655	17.3283	20.3326	< 0.01
	Time	0.0019	0.0003	0.0013	0.0025	< 0.01
	HIV-HCV-3	1.7684	1.4574	-4.6856	1.1488	0.23

HIV, human immunodeficiency virus; HBV, hepatitis B virus; HCV, hepatitis C virus; SE, standard error; LI, inferior limit; LS, upper limit; The linear mixed-effects model was used to compare the CD4 cell counts between groups.

Table 3. Estimate with standard errors for the fixed effects parameters of the model for CD4/CD8 ratio.

Group	Parameters	Estimates	SE	LI	LU	p-valor
HIV-HBV	Intercept	0.6149	0.0216	0.5725	0.6573	< 0.01
	Time	0.0002	0.0001	0.0001	0.0002	< 0.01
	HIV-HBV	0.0395	0.0430	-0.0454	0.1244	0.36
HIV-HCV	Intercept	0.6655	0.0215	0.6233	0.6655	< 0.01
	Time	0.0001	0.0001	0.0001	0.0001	< 0.01
	HIV-HCV	-0.0433	0.0401	-0.1225	0.0360	0.28
HIV-HCV-3	Intercept	0.6630	0.0327	0.5988	0.7272	< 0.01
	Time	0.00005	0.0001	0.0002	0.0007	< 0.01
	HIV-HCV-3	-0.1256	0.0595	-0.2447	-0.0065	0.04

HIV, human immunodeficiency virus; HBV, hepatitis B virus; HCV, hepatitis C virus; SE, standard error; LI, inferior limit; LU, upper limit. The linear mixed-effects model was used to compare the CD4/CD8 ratio between groups.

Table 4. Multivariate logistic analyses of HIV viral load.

Group	Parameters	OR	CI 95%	p-value
HIV-HBV	Intercept	1.8704	1.2596-2.7774	< 0.01
	Time	0.9983	0.9981-0.9986	< 0.01
	HIV-HBV	1.1442	0.6412-2.0418	0.65
HIV-HCV	Intercept	1.8234	1.1533-2.8828	< 0.01
	Time	0.9988	0.9986-0.9990	< 0.01
	HIV-HCV	1.5204	0.8193-2.8215	0.18
HIV-HCV-3	Intercept	15.2567	8.9569-25.9877	< 0.01
	Time	0.9979	0.9966-0.9991	< 0.01
	HIV-HCV-3	0.8117	0.3425-1.9493	0.54

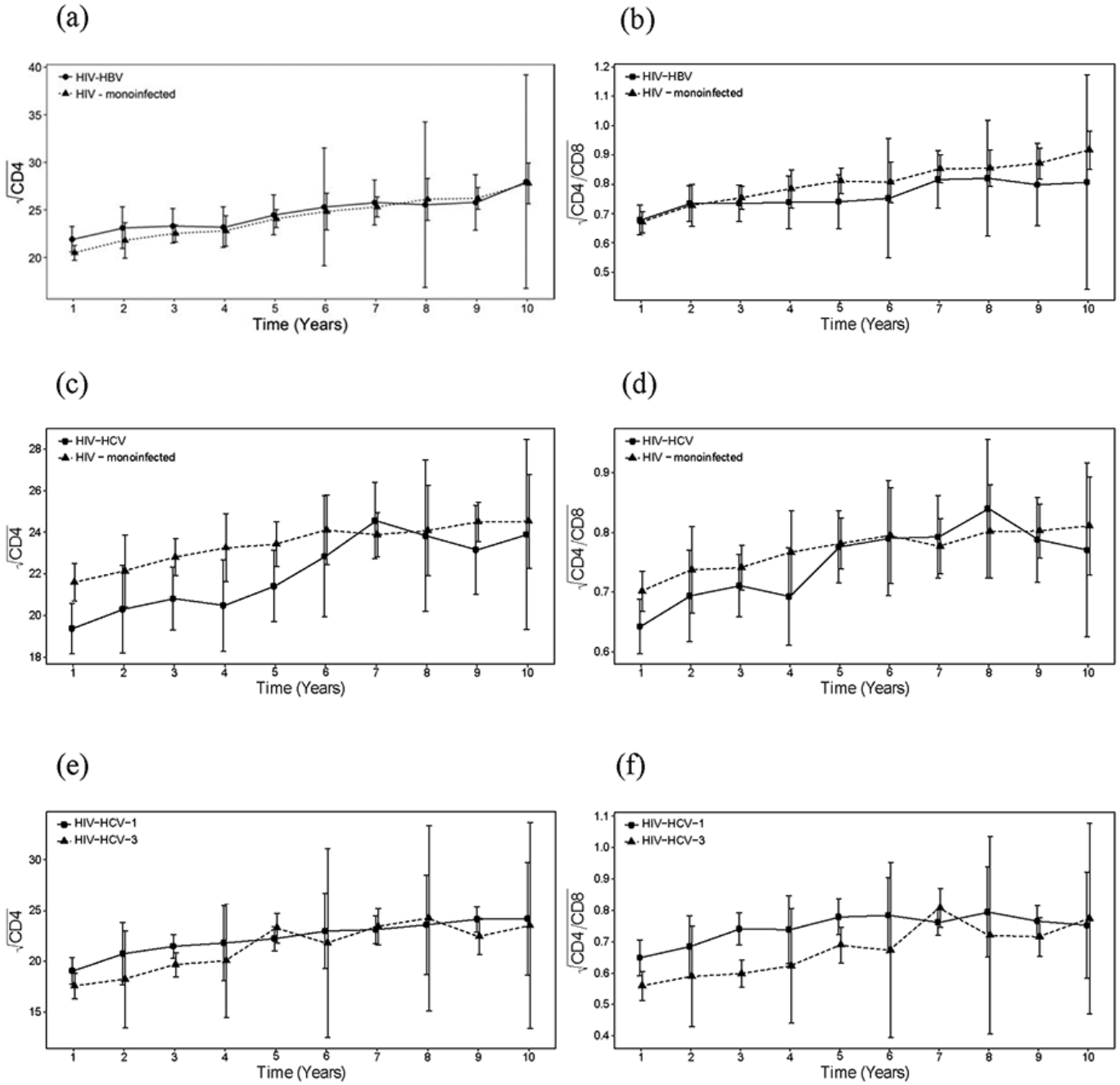
HIV, human immunodeficiency virus; HBV, hepatitis B virus; HCV, hepatitis C virus; OR, odds ratio; CI, confidence interval. The mixed logistic model was used to compare the HIV viral load categorized between groups.

The mean square root of CD4 cell counts, CD4/CD8 ratios for the study groups over time (years) are shown in Figure 1.

Discussion

A retrospective analysis was performed by evaluating CD4 cell counts and the CD4/CD8 ratio over years using a linear mixed-effects model. These models are suitable for the analysis of grouped and hierarchical longitudinal data and permit the description and

Figure 1. Square root CD4 cell and CD4/CD8 ratio over time (years) for groups studied.



HIV, human immunodeficiency virus; HBV, hepatitis B virus; HCV, hepatitis C virus; Evolution CD4 cell count and CD4/CD8 ratio during follow-up was estimated using a linear mixed-effects model; (a) Square root CD4 cell count of HIV monoinfection when compared to HIV-HBV coinfection; (b) Square root CD4/CD8 ratio of HIV monoinfection when compared to HIV-HBV coinfection; (c) Square root CD4 cell count of HIV monoinfection when compared to HIV-HCV coinfection; (d) Square root CD4/CD8 ratio of HIV monoinfection when compared to HIV-HCV coinfection; (e) Square root CD4 cell count of HCV genotype 1 when compared to HCV genotype 3 among HIV infection; (f) Square root CD4/CD8 ratio of HCV genotype 1 when compared to HCV genotype 3 among HIV infection.

comparison between average profiles of responses, taking into account the variability and interaction between experimental units of the same group and values observed in these units [28]. In addition, mixed-effects models have the potential to develop into clinical tools. These clinical tools may predict the CD4 cell count of patients at follow-up visits and are therefore potentially helpful in the identification of patients at risk of rapid progression [28]. In this study, the variable of Time was significant for the recovery of CD4 cell count and increase of CD4/CD8 ratio in all groups receiving cART. Improved CD4 cell count in response to cART is the result of viral suppression, which allows the body's homeostatic mechanisms to repopulate the CD4 cell pool [29].

There was no statistically significant difference in CD4 cell counts and the CD4/CD8 ratio between HIV monoinfection and HIV-HBV-coinfected groups during follow-up on cART. Previous studies have not found evidence that HBV accelerates or aggravates the natural history of HIV infection [30,31]; our study corroborates these findings. However, there was a significantly lower CD4 cell count in patients with HIV-HCV coinfection during follow-up on cART than in patients with HIV monoinfection. This can likely be attributed to higher pre-ART levels of CD4 cell counts in the HIV monoinfection group than in the HIV-HCV group. Alternatively, HCV infection could impact the course of HIV infection via chronic immune activation and cytokine production in coinfected individuals [32,33], which may result in diminished CD4 cell counts [34]. Coinfection with HCV has also been associated with increased CD4 cell apoptosis [35] and can cause damage to the immune system, thus leading to a subsequent increase of viral replication of HIV and HCV, further contributing to an impaired immune system and consequently lower CD4 cell counts [36,37]. In addition, significantly elevated CD38 expression in both CD8 and CD4 cells has been described in HIV-HCV coinfection, indicating that coinfection is associated with high levels of chronic activation of both T-cell compartments [33]. Chronic T-cell activation related to HCV infection may limit the immunological responses of patients on cART [38].

The natural history of HIV infection is characterized by a progressive decrease in CD4 cells and a parallel increase in CD8 cells, leading to an inverted CD4/CD8 ratio, generally within less than one year after seroconversion [39]. In the present study, patients with HCV coinfection showed lower CD4/CD8 ratios than patients with HIV monoinfection during follow-up on cART; however, the difference was not statistically significant. The same trend was observed for the HIV-HBV group. In addition, a progressive increase of the CD4/CD8 ratio was still observed during cART in HIV monoinfection patients, as well as in HBV- and HCV-coinfected groups, although this was lower than that of HIV monoinfection patients. We also observed a lower recovery of the CD4/CD8 ratio in HIV-HBV patients compared with HIV patients. This is clinically relevant as studies have demonstrated that failure to normalize the CD4/CD8 ratio under cART increases the risk of incidence of non-AIDS related events, independently of CD4 cell counts [40]. Some factors may influence the low CD4/CD8 ratio observed in HIV-HBV-coinfected patients during the follow-up on cART, such as association with chronic hepatitis B that may lead to immune activation, which increases CD4 cell apoptosis [41].

Different HCV genotypes and duration of infection are associated with the progression of liver disease [42]. The present study showed a lack of statistically significant difference between HCV genotypes 1 and 3 among HIV-infected patients for the CD4 cell count in pre-ART and during follow-up on cART; these data are also supported by the literature [43,44]. However, the CD4/CD8 ratio was lower for HCV genotype 3 than for genotype 1 during follow-up on cART ($p = 0.04$). A few studies have systematically investigated the CD4/CD8 ratio among HCV genotypes in HIV-infected patients. In a study performed in Brazil, no statistically significant differences were observed in CD4/CD8 ratio between HCV genotypes 1 and 3 among HIV-infected patients [44]. However, patients infected with HCV genotype 3 showed faster liver fibrosis progression, often accompanied by liver steatosis, and HCV genotype 3 was related to prediction factors for severe hepatic fibrosis (F3-F4) [42,45]. Interestingly, patients with F3-F4 liver fibrosis demonstrated lower CD4/CD8 ratios compared with patients who had F0-F2 fibrosis [46].

Our study has some limitations. First, although the study contemplates the period from January 2002 to June 2016, a few HBV- and HCV-coinfected patients were followed up for more than five years. Owing to this factor, for the Time variable, there were considerable variations in CD4 cell counts and the CD4/CD8 ratio. Second, it was not possible to determine the time of coinfection in pre-ART. Furthermore, there was no information about the degree of liver dysfunction in our patients. It is possible that underlying liver dysfunction may be related to the changes in CD4 cell counts. Third, because of few and unmatched patients and no adherence to cART during

follow-up, the power of the study may be insufficient to compare the immunological impact between HCV genotypes 1 and 3 on HIV-infected patients, which may be masked by potential confounders; this may prevent us from reaching more precise conclusions. However, our results are consistent with those from other studies and enhance our knowledge on HIV-infected patients and coinfection with HBV or HCV.

Conclusions

HBV coinfection in HIV-infected patients did not have a significant impact on CD4 cell count and CD4/CD8 ratio in pre-ART and during follow-up on cART. However, the CD4 cell count was significantly lower for patients with HIV-HCV coinfection compared with those who had HIV monoinfection during follow-up on cART. These findings suggest that HIV-infected patients are more likely to have a better immunological response to cART, if they are not coinfected with HCV. Prospective cohort studies should be conducted that account for multiple variables, such as the degree of liver disease, duration of HIV and HCV infection, and different treatment regimens, for better understanding of the effect of HCV in HIV-infected patients on cART.

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Authors' contributions

Silva CM conceived the study, participated in its design and coordination, and drafted the manuscript. Peder LD was responsible for data collection and participated in the study design. Silva ES, Previdelli I and Pereira OCN performed the statistical analysis. Teixeira JJV and Bertolini DA participated in the interpretation of data and revised the paper critically. All authors read and approved the final manuscript.

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